Orderly recruitment of thermoeffectors in resting humans

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Abstract

The recruitment of thermoeffectors, including thermoregulatory behavior, relative to changes in body temperature has not been quantified in humans. We tested the hypothesis that changes in skin blood flow, behavior, and sweating or metabolic rate are initiated with increasing changes in mean skin temperature (T_{skin}) in resting humans. While wearing a water perfused suit, twelve healthy young adults underwent heat (HEAT) and cold (COLD) stress that induced gradual changes in T_{skin}. Subjects controlled the temperature of their dorsal neck to their perceived thermal comfort. Thus, neck skin temperature provided an index of thermoregulatory behavior. Neck skin temperature, T_{skin}, core temperature (T_{core}), metabolic rate, sweat rate, and non-glabrous skin blood flow were measured continually. Data were analyzed using segmental regression analysis, providing an index of thermoeffector activation relative to changes in T_{skin}. In HEAT, increases in skin blood flow were observed with the smallest elevations in T_{skin} ($P<0.01$). Thermal behavior was initiated with an increase in T_{skin} of 2.4 ± 1.3°C ($P=0.04$), while sweating was observed with further elevations in T_{skin} (3.4 ± 0.5°C, $P=0.04$), which coincided with increases in T_{core} ($P=0.98$). In COLD, reductions in skin blood flow occurred with the smallest decrease in T_{skin} ($P<0.01$). Thermal behavior was initiated with a T_{skin} decrease of 1.5 ± 1.3°C, while metabolic rate ($P=0.10$) and T_{core} ($P=0.76$) did not change throughout. These data indicate that autonomic and behavioral thermoeffectors are recruited in coordination with one another and likely in an orderly manner relative to the comparative physiological cost.

Keywords: Metabolic heat production, Sweat rate, Thermoregulatory behavior, Skin blood flow, Thermal discomfort

Abbreviations:

T_{skin}: mean skin temperature, T_{core}: core temperature, HEAT: heat stress trial, COLD: cold stress trial, Breakpoint: point at which there is an abrupt change in the dependent variable as a function of the independent variable, Slope1: slope of the regression line before the Breakpoint, Slope2: slope of the regression line after the Breakpoint, CVC: cutaneous vascular conductance, T_{neck}: neck skin temperature, SkBF: skin blood flow, LSR: local sweat rate, Δ: change.

Introduction

Temperature regulation is achieved via autonomic and behavioral thermoeffectors (42). Thermoeffector responses are generally believed to be recruited at distinct deviations in mean skin (T_{skin}) and/or core (T_{core}) body temperatures (13). For instance, it is often posited that sweating is recruited at higher body temperatures than cutaneous vasodilation during heat stress, and changes in metabolism at lower body temperatures than cutaneous vasoconstriction during cold stress (22). This is termed orderly recruitment (35) such that thermoeffectors are initiated relative to their comparative physiological cost, with changes in skin blood flow utilizing less resources than sweating (e.g., water and electrolytes) or increases in metabolism (e.g., energy) (22, 32). With this in mind, it can be reasonably posited that thermal behavior is elicited by greater changes in body temperature compared to skin blood flow, but less extreme changes in body temperature compared to sweating or changes in metabolism. However, it is unknown if thermal behavior is recruited in an orderly manner and in coordination with autonomic thermoeffectors in humans.

We have found that when given the opportunity to behaviorally thermoregulate, the initiation of thermal behavior is mediated by changes in T_{skin}, preventing changes in T_{core} (39, 40). We have also identified that the initiation of thermal behavior temporally precedes the initiation of sweating in the heat and changes in metabolism in the cold (38). However, thermal behavior is consistently preceded by relatively profound changes in skin blood flow (38, 41). These temporal observations suggest that behavior is recruited in an orderly manner and in coordination with autonomic thermoeffectors. However, temperature regulation is not dependent on time, per se, but rather, is determined by afferent thermosensory feedback (22). Thus, examining the thermoeffector responses relative to changes in body temperature permits unique insights regarding the relative control of thermoeffectors. However, the recruitment of autonomic and behavioral thermoeffectors in humans relative to changes in body temperature has not been quantified. Therefore, the purpose of this study was to test the following hypotheses: i) changes in skin blood flow would be elicited by the smallest deviations in T_{skin}, ii) further changes in T_{skin} would bring about the initiation of thermal behavior, and iii) sweating or changes in metabolism would be elicited by the largest changes in T_{skin}, and coinciding more closely with changes in T_{core}, in resting humans.

Methods

Subjects

Thirteen healthy young adults participated in this study. The subject characteristics were: age: 22 ± 2 y, height: 170 ± 8 cm, weight: 70.6 ± 10.7 kg, body surface area: 1.8 ± 0.2 m^2, percent body fat: 25 ± 14%, 7 females. Subjects were physically active, normotensive, non-smokers, not taking medications, and cognitively normal. Subjects were free from any known cardiovascular, metabolic, psychological, or neurological diseases, and reported normal thermal sensation. Female subjects were not pregnant, which was confirmed via a urine pregnancy test, and self-reported to be normally menstruating. Four of the female subjects were taking oral contraceptives. Each subject was fully informed of the experimental procedures and possible risks before giving informed written consent. The study was approved by the Institutional Review Board at the University at Buffalo, and performed in accordance with the standards set by the latest revision of the Declaration of Helsinki.

Study design

Subjects visited the laboratory on three occasions. Visit one was a screening and familiarization visit and visits two and three were the experimental trials. The experimental trials involved either progressive heat stress (HEAT) or cold stress (COLD). The order of these trials was randomized. For the experimental trials, subjects arrived at the laboratory euhydrated, confirmed via urine specific gravity ≤1.020 (36) (HEAT: 1.012 ± 0.007, COLD: 1.009 ± 0.007, P=0.41), and having refrained from strenuous exercise, alcohol and caffeine for 12 h, and food for 2 h. To control for menstrual cycle hormones, females were tested during the first 10 days following self-identified menstruation (n=3) or during the placebo phase of their oral contraceptives (n=4), a period in which estrogen and progesterone are at their lowest levels (13). All experimental testing was conducted during the spring and summer months in Buffalo, NY, USA. The outdoor temperature on day and time of the experimental trials was 14 ± 9°C. However, it was assumed that all subjects were not heat acclimatized given the absence of evidence of seasonal heat acclimatization in a humid continental climate (1). For each subject, both experimental trials were completed at the same time of day to control for variations in temperature regulation associated with circadian rhythms (6).

Instrumentation and measurements

Height and weight were measured with a stadiometer and scale (Sartorius Corp. Bohemia, NY, USA), and body surface area was calculated accordingly (12). Urine specific gravity was measured in duplicate using a refractometer (Atago USA, Inc., Bellevue, WA, USA). Physical activity level was estimated using the validated International Physical Activity Questionnaire (9) and cognitive ability was measured using the Montreal Cognitive Assessment (27). Skinfold thickness was measured in triplicate at the chest, axilla, triceps, subscapula, abdomen, suprailliac, and thigh (Harpenden, Baty International, UK), and percent body fat was estimated from body density (44), which was calculated from the sum skinfolds for males (19) and females (20).

Body temperature was controlled via a tube-lined water perfused suit (Med-Eng, Ottawa, ON, Canada) that covered the entire body except the head, hands, and feet. At least 60 min prior to any experimental testing, subjects swallowed a telemetry pill (HQ Inc., Palmetto, FL, USA) for the measurement of T_{core}. One female subject had contraindications for taking the telemetry pill. In this subject, rectal temperature was measured at a depth of 10 cm past the anal sphincter using a general purpose thermistor (Mon-a-therm, Mallinckrodt Medical, Inc., St. Louis, MO, USA). T_{skin} was measured under the water perfused suit as the weighted average of six thermocouples (Omega Engineering, Inc. Stamford, CT, USA) attached to the following locations: abdomen (14%), calf (11%), chest (22%), lower back (19%), thigh (14%), and upper back (20%) (47).

Heart rate was continually measured via a 3-lead ECG (DA100C, Biopac Systems, Inc. Goleta, CA, USA). Beat-to-beat blood pressure was measured via the Penaz method (Finometer Pro, FMS, Amsterdam, The Netherlands), which was verified intermittently via electrosphygmomanometry (Tango M2, SunTech, Raleigh, NC, USA) of the brachial artery. Finometer derived blood pressure waveforms were maintained throughout all experimental testing except in one COLD trial. In this trial, brachial artery blood pressure was used in the analysis.

Skin blood flow was measured via integrated laser Doppler flowmetry (Periflux System 5010, Perimed, Stockholm, Sweden) on the dorsal surface of the left upper arm under the water-perfused suit. This non-glabrous skin location was chosen because it was under the water perfused suit and is more centrally located than the forearm, but not as readily influenced by changes in ventilation as location on the trunk (e.g., chest). The local temperature of the upper arm location was measured continually (Periflux System 5020, Perimed, Stockholm, Sweden). The accuracy of the skin blood flow measurement was ensured

based upon the observation of a clear pulsatile signal that coincided with the pulse wave. Skin blood flow data were also normalized to mean arterial pressure, providing an index of cutaneous vascular conductance (CVC), which is indicative of changes in cutaneous vasomotor tone. However, given that heat transfer is directly dependent on the absolute volume of blood perfusing the skin, changes in skin blood flow (not CVC) were interpreted as the thermoeffector response.

Local sweat rate was measured by securing a plastic capsule that covered 3.9 cm^2 of skin on the dorsal surface of the left upper arm under the water perfused suit, directly adjacent to the skin blood flow measurement location. This site was chosen because of the proximity to the skin blood flow measurement location, which better permitted comparisons in the local sweat rate and skin blood flow responses. The capsule was perfused with dry nitrogen at a flow rate of 0.6 L/min. The water vapor of the gas exiting the capsules was measured by capacitance hygrometry (HMT130, Vaisala, Woburn, WA, USA), and local sweat rate was calculated by multiplying the humidity output by flow rate and dividing that value by the surface area of the capsule (16).

Skin blood flow and local sweat rate were measured under the water perfused suit to quantify the recruitment of these thermoeffectors independent of the mechanism by which they were recruited (i.e., local vs. reflex). This is an important consideration because the purpose of this study was not to identify the control mechanisms, but rather to quantify the order in which these thermoeffector responses were recruited relative to changes in T_{skin}.

Metabolic data were obtained via a facemask and three-way non-rebreathing valve (Han Rudolph, Inc., Shawnee, KS, USA) that was worn throughout the study. The rate of metabolic heat production was calculated from oxygen uptake and the respiratory exchange ratio (RER) and normalized to body surface area using a standard equation (10). Oxygen uptake and carbon dioxide production were calculated from minute ventilation and the fraction of expired oxygen and carbon dioxide using the Haldane Transformation. Minute ventilation was calculated from expired airflow that was measured via a heated pneumotachometer (Hans Rudolph, Inc. Shawnee, KS, USA), which was continually integrated over 30 s and corrected to STPD. The fraction of expired oxygen and carbon dioxide (Vacumed, Ventura, CA, USA) was continually measured from a 3 L mixing chamber.

Quantification of thermal behavior

Thermal behavior was measured using techniques modified from those of Cabanac et al. (4, 5) during which subjects were instructed to control the temperature of their hand so that it was thermally comfortable throughout the experiments. The only difference was that in the present study subjects were instructed to control the temperature of the dorsal aspect of their neck. The neck was chosen because it is the only skin area known to be equally and highly sensitive to both cooling and heating stimuli (26). Thus, neck skin temperature provided an objective and continuous index of thermal behavior (4, 5). Neck skin temperature was measured with a single thermocouple (Omega Engineering, Inc. Stamford, CT, USA) secured to the center of the dorsal aspect of the neck.

Neck skin temperature was controlled using a dual tubing system that was placed in direct contact with the subject's neck. The Tygon tubing system contained two unique series of tubing (inner diameter: 7.94 mm, outer diameter: 9.52 mm). One series included four 8 cm lengths positioned in parallel. This series was continually perfused with 35°C water at a constant flow rate of 2.2 L/min. The second series of tubing included five 8 cm lengths positioned in parallel, alternating with the first series. The temperature of the fluid perfusing the second series differed depending on the trial. During HEAT, when it was expected that subjects would want to cool their neck, the second series was perfused by -20°C fluid (antifreeze), while during COLD, when it was expected that subjects would want to heat their neck, the second series was perfused by 52°C fluid. The flow rate of the second series was directly controlled by the subject via a two-way ball valve, permitting flow rates of 0-2.2 L/min. Thus, by controlling the flow of the second series subjects were permitted to behaviorally thermoregulate. Subjects controlled the valve with their right hand and were well familiarized with, and practiced using, the dual tubing system before any experimental testing. Subjects were instructed at the start of each trial to keep their neck at a comfortable temperature regardless of how the rest of their body felt.

Based on extensive pilot testing, this dual tubing system was found to allow for a range of neck skin temperatures to be rapidly achieved, with the perception of changes in neck skin temperature being sensed within 15-20 s of adjusting the valve. Furthermore, this dual tubing system also ensured subjects were constantly adjusting the temperature of their neck to maintain thermal comfort. This allowed for quantification of both the initiation and magnitude of the behavioral responses, and provided an advantage over the binary (i.e., yes-no) quantification of thermal behavior that other models (e.g., shuttle box) usually produce (40).

Finally, this model allowed for quantification of thermal behavior without the need for the subject to physically move.

All subjects behaviorally thermoregulated, as determined by changes in neck skin temperature, except one female who did not behaviorally thermoregulate in either HEAT or COLD. The reason for this is unknown. We speculate, however, that despite thorough familiarization, this may have been due to misunderstanding of the use of the dual tubing system. Nevertheless, this subject was excluded from the data analysis resulting in n=12. The subject characteristics are presented in Table 1.

Experimental protocols

Following instrumentation, subjects rested quietly in the supine position for at least 20 min with 34°C water perfusing the suit. Following this period, the subject underwent 60 min of either progressive heat stress (HEAT) or cold stress (COLD). In HEAT, the temperature of the water perfusing the suit was increased by 2°C every 4 min until 50°C water was perfusing the suit. This temperature was then maintained for the remainder of the 60 min time period (Figure 1A). During HEAT, subjects were wrapped in a Mylar blanket (Primacare Medical Supplies, Passaie, NJ, USA) to help prevent evaporative heat loss and promote heat gain. During COLD, the temperature of the water perfusing the suit was decreased by 2°C every 4 min until 14°C water was perfusing the suit. This temperature was then maintained for the remainder of the 60 min time period (Figure 1D). All trials were completed in a temperature controlled laboratory (24 $\pm$ 1°C).

These heating and cooling protocols were employed to first induce gradual and progressive changes in T_{skin} and eventually changes in T_{core}. Because changes in cutaneous vasomotor tone and thermal behavior during rest are observed prior to meaningful changes in T_{core} (39), these protocols were designed to identify the distinct changes in T_{skin} associated with the onset of autonomic and behavioral thermoeffectors and changes in T_{core}.

Data and statistical analyses

Data were sampled continuously at 100 Hz via a data acquisition system (Biopac MP150, Goleta, CA, USA). Two analyses were undertaken. First, data were reduced to 60 s averages. Data at baseline (time = 0 min) and every 4 min thereafter were analyzed as both absolute values and as a change from baseline, accounting for any between-subject differences at baseline. These data were analyzed using a two-way repeated measures ANOVA (trial x time).

Second, changes from baseline in the thermoeffector and T_{core} data were reduced to 10 s averages and were plotted over changes in T_{skin}. These data were analyzed using segmental regression (7). This analysis provided three parameters: i) Slope1, which is indicative of the slope of the regression line before the breakpoint; ii) Breakpoint, which is indicative of the point at which there is an abrupt change in the dependent variable as a function of T_{skin}; and iii) Slope2, which is indicative of the slope of the regression line after the breakpoint (Figure 2). All of the aforementioned analyses were carried out a priori. However, post hoc inspection of data during COLD revealed that T_{core} (Figure 2E) and metabolic heat production (Figure 2G) data did not fit the segmented regression model (i.e., a breakpoint could not be identified). This was because neither T_{core} (Figure 1F) or metabolic heat production (Figure 3G) changed during COLD. As a result, the results of the segmental regression analyses for these data were omitted. During HEAT, the Breakpoints for the thermoeffector data (neck skin temperature, skin blood flow, and local sweat rate) were compared using a one-way repeated measures ANOVA. During COLD, the Breakpoints for the thermoeffector data (neck skin temperature and skin blood flow) were compared using paired t-tests. Slope1 and Slope2 were analyzed for deviation from zero (i.e., no slope) using single sample t-tests, providing an indication of thermoeffector activation prior to and after the breakpoint. Slope1 and Slope2 were also normalized to the maximum change in a given thermoeffector within a subject and trial. The resulting relative slopes (i.e., Relative Slope1, Relative Slope2) were compared using a one-way repeated measures ANOVA. This relative slope analysis allowed for insights regarding the comparative extent of activation between thermoeffectors. In a separate analysis, the breakpoints for T_{core} were compared with those of the thermoeffectors using a one-way repeated measures ANOVA and Dunnett's test for multiple comparisons. This provided insights into any temporal differences between changes in T_{core} and thermoeffector activation in HEAT.

Unless stated otherwise, post hoc pairwise comparisons were made using Tukey's test. All data were analyzed using Prism software (Version 6, GraphPad Software Inc. La Jolla, CA, USA). A priori statistical significance was set at $P \leq 0.05$. Actual P-values are reported where possible. Data are reported as mean ± SD.

Results

Body temperatures

In HEAT, progressively increasing the temperature of water perfusing the water perfused suit raised T_{skin} by 3.7 ± 0.4°C through 36 min (P<0.01), with an increase in T_{skin} of 4.1 ± 0.2°C being maintained through the end of the trial (Figure 1B). Elevations T_{core} were observed at 36 min, with T_{core} increasing by 0.9 ± 0.3°C by the end of the 60 min trial (P<0.01, Figure 1C).

In COLD, progressively decreasing the temperature of the water perfusing the water perfused suit decreased T_{skin} throughout such that at 60 min T_{skin} had dropped by 6.0 ± 0.6°C (P<0.01, Figure 1E). T_{core} did not change throughout COLD (P=0.10, Figure 1F).

Temporal thermoregulatory responses

Increases in skin blood flow (P=0.02, Figure 3A) and CVC (P=0.01, Figure 3B) occurred by the 12th min of progressive heat stress. By contrast, in HEAT increases in local sweat rate (Figure 3C) and decreases in neck skin temperature (Figure 3D) occurred at the 36th min (P=0.056) and 40th min (P=0.03). Maximal decreases in neck skin temperature were 7.9 ± 4.6°C (min: -0.9°C, max: -14.3°C). Mean arterial pressure did not change throughout HEAT (0 min: 80 ± 8 mmHg, 60 min: 77 ± 6 mmHg, P=0.21). Increases in heart rate occurred by the 28th min of progressive heat stress (by 10 ± 5 bpm, P<0.01) and continued to increase thereafter (increase at 60 min: 41 ± 10 bpm, P<0.01).

Reductions in skin blood flow (P=0.03, Figure 3E) and CVC (P<0.01, Figure 3F) occurred by the 16th min of progressive cold stress. In COLD, neck skin temperature did not differ from baseline until the 32nd min (P=0.01, Figure 3H), while metabolic heat production did not change throughout (P=0.76, Figure 3G). Maximal increases in neck skin temperature were 4.3 ± 1.9°C (min: +0.8°C, max: +7.2°C). Elevations in mean arterial pressure occurred by the 12th min of progressive cold stress (by 4 ± 3 mmHg, P=0.03) and continued to increase thereafter (increase at 60 min: 14 ± 4 mmHg, P<0.01). Heart rate did not change throughout COLD (0 min: 63 ± 12 bpm, 60 min: 61 ± 6 bpm, P=0.25).

Segmental regression analysis

An example of the segmental regression analysis during HEAT is noted in Figure 2. In HEAT, skin blood flow deviated from zero before the Breakpoint (Slope1). Slope1 for neck skin temperature demonstrated a slightly positive slope when expressed relative to T_{skin} (P<0.01,

311 Table 2). Notably, however, this was in the opposite direction of what would be expected if
312 subjects had behaviorally thermoregulated. Slope1 for local sweat rate did not deviate from
313 zero (P=0.08, Table 2). Relative Slope1 for skin blood flow was greater than local sweat rate
314 and neck skin temperature (P≤0.03) and Relative Slope1 for neck skin temperature did not
315 differ from that of local sweat rate (P≥0.08, Figure 4A). In HEAT, the T_{skin} Breakpoint for local
316 sweat rate was higher than the Breakpoints for neck skin temperature (P<0.01) and skin blood
317 flow (P=0.04, Figure 4B). Moreover, the T_{skin} Breakpoint for neck skin temperature was lower
318 than the Breakpoint for skin blood flow (P=0.04, Figure 4B). In HEAT, changes in activation
319 after the Breakpoint (Slope2) differed from zero for all thermoeffectors (P≤0.03, Table 2).
320 Furthermore, the relative extent of activation (Relative Slope2) did not differ between
321 thermoeffectors (P=0.33, Figure 4C). In HEAT, the T_{skin} Breakpoint for T_{core} (3.5 $\pm$ 0.4°C) was
322 higher than the Breakpoint for neck skin temperature (P=0.02), but was not different from that
323 for skin blood flow (P=0.09) or local sweat rate (P=0.98).
324 An example of the segmental regression analysis during COLD is noted in Figure 2. In
325 COLD, Slope1 for neck skin temperature did not deviate from zero (P=0.33, Table 2).
326 However, Slope1 for skin blood flow was different from zero (P<0.01, Table 2). Relative Slope1
327 for skin blood flow was greater than neck skin temperature (P<0.01, Figure 4D). In COLD, the
328 T_{skin} Breakpoint for skin blood flow and neck skin temperature was not different (P=0.61,
329 Figure 4E). In COLD, Slope2 differed from zero for both thermoeffectors (P<0.01, Table 2).
330 Relative Slope2 was higher for neck skin temperature compared to skin blood flow (P<0.01,
331 Figure 4F).

Discussion

The goal of the present study was to determine the orderly recruitment of autonomic and behavioral thermoeffectors relative to progressive changes in T_{skin} in resting humans. There are a number of novel findings. First, during progressive heat stress the T_{skin} threshold upon which thermal behavior was initiated (i.e., the Breakpoint) was lower than that for local sweat rate and non-glabrous skin blood flow, while the T_{skin} Breakpoint for increases in non-glabrous skin blood flow was between that for thermal behavior and local sweat rate (Figure 4B). Second, increases in skin blood flow were observed before the skin blood flow T_{skin} Breakpoint during heat stress (Figure 4A, Table 2). Third, during progressive cold stress the T_{skin} Breakpoint for thermal behavior coincided with the skin blood flow T_{skin} Breakpoint (Figure 4E). Fourth, skin blood flow did not demonstrate a vasoconstrictor activation threshold during cold stress, but rather a continuous vasoconstriction and an eventual leveling off after the Breakpoint (Figure 4F, Table 2). Collectively, our data indicate that during progressive heat and cold stress autonomic and behavioral thermoeffectors are recruited in coordination with one another in resting humans. In the context of the current study, these findings suggest that thermoeffector recruitment occurs in an orderly manner relative to the comparative cost, with thermal behavior requiring more physiological resources (e.g., energy) compared to changes in skin blood flow, yet less than sweating (e.g., water) or increases in metabolism (e.g., energy).

Thermoeffector recruitment during progressive heat stress

During heat stress, it is believed that thermoeffectors are recruited to preserve body water and electrolytes that may be lost due to sweating (32). In support of this idea, increases in skin blood flow temporally precede sweating, while behavior is initiated after skin blood flow has increased, but before the onset of sweating (38). Notably, however, temperature regulation is not dependent on time, per se, but rather, is determined by afferent feedback from both peripheral (i.e., T_{skin}) and central (i.e., T_{core}) thermosensors (22). Thus, examining the thermoeffector responses relative to T_{skin}, an index of afferent feedback and the primary independent variable in the present study, permits unique insights regarding the relative control of thermoeffectors. In this regard, the present study has identified that the initiation of thermal behavior occurs at lower T_{skin} than the Breakpoints for either skin blood flow or sweating (Figure 4B). Furthermore, only the T_{skin} Breakpoints for skin blood flow and sweating coincided with increases in T_{core}. Moreover, skin blood flow continually increased both before

and after the T_{skin} Breakpoint such that both Slope1 and Slope2 were positive and significantly different from exhibiting no slope (Table 2, Figure 4A and 4C). This skin blood flow response is clearly unlike the thermal behavior and sweating responses, which did not reflect any activation before their respective Breakpoints (Figure 4A). Furthermore, the relative extent of activation after the Breakpoints (i.e., Relative Slope2) did not differ between thermoeffectors (Figure 4C). Collectively, these findings support our hypothesis that increases in skin blood flow are elicited by the smallest increases in T_{skin} and that continued elevations in T_{skin} elicit thermal behavior and eventually sweating, the latter of which more closely coincides with changes in T_{core}.

Thermoeffector recruitment during progressive cold stress

During cold stress, thermoeffectors are thought to be recruited to conserve the energy that would be consumed by increases in metabolic rate associated with shivering and/or non-shivering thermogenesis (32). In support of this, we have previously demonstrated that reductions in skin blood flow temporally precede shivering, while behavior is initiated after skin blood flow has decreased, but before any changes in metabolic rate (38). The present study extends these findings in that the initiation of thermal behavior and the leveling off of skin blood flow occurred after the same reduction in T_{skin} (Figure 4E). As a result, the relative extent of activation following the Breakpoint (i.e., Relative Slope2) was greater for thermal behavior compared to skin blood flow (Figure 4F), suggesting that there was an inability to further vasoconstrict and a comparatively greater ability to behaviorally thermoregulate. These findings support our hypothesis that decreases in skin blood flow are elicited by the smallest reductions in T_{skin} and that further drops in T_{skin} initiate thermal behavior.

Unexpectedly, we did not observe any changes in T_{core} (Figure 1F) or metabolic rate (Figure 3G) during progressive cold stress. It is likely that our water perfused suit model, in which the hands, feet and face were not cold stressed, may have resulted in an insufficient rate of heat loss to reduce T_{core} within 60 min. Because of the contribution that reductions in T_{core} play in the shivering response (17), that T_{core} did not change may have contributed to why we did not observe any changes in metabolic rate. That said, T_{skin} also plays an important role in modifying metabolic rate during cold stress before reductions in T_{core} (17). However, the rate of change in T_{skin} likely modulates this response (2). Thus, the gradual reductions in T_{skin} induced by our progressive cold stress protocol may have also contributed to the absence of any changes in metabolic rate. It is unfortunate that we were unable to test our hypothesis that

increases in metabolic rate would be initiated at greater reductions in T_{skin} compared to both skin blood flow and thermal behavior and that these changes in metabolic rate would coincide more closely with changes in T_{core}. Although such a hypothesis seems reasonable, direct evidence is required.

Thermal behavior relative to the thermoeffector threshold zone

The thermoeffector threshold zone is defined as the temperature range between two threshold body temperatures for activation of metabolic heat production and sweating (24). Thermal behavior was initiated at a cooler T_{skin} in the heat compared to sweating (Figure 4B) and a warmer T_{skin} in the cold compared to changes in metabolism (Figures 3G, 4E). Thus, the present study has clearly demonstrated that thermal behavior is elicited within the body temperature ranges that define the thermoeffector threshold zone. This supports previous data demonstrating that sweating or changes in metabolism are not required for the initiation of thermal behavior (38). This suggests that when resting humans are free to behaviorally thermoregulate, sweating or metabolic changes are not the signal mediating the decision to behave.

The present study has also identified unique interactions between the control of thermal behavior and skin blood flow within the thermoeffector threshold zone. For instance, it is likely that the increase in skin blood flow prior to the T_{skin} Breakpoint during progressive heat stress is indicative of the withdrawal of cutaneous sympathetic vasoconstrictor tone (29, 46), although in the current study a role for local mechanisms cannot be excluded (28). Furthermore, the T_{skin} Breakpoint and the gain of the skin blood flow to T_{skin} relationship thereafter (i.e., Slope2) is likely a function of the onset and magnitude of sympathetic active cutaneous vasodilation (29, 46). Previous observations indicate that the initiation of thermal behavior during heat stress temporally coincides with active cutaneous vasodilation (38). The present study extends these findings such that thermal behavior was elicited at lower T_{skin} compared to the T_{skin} Breakpoint for skin blood flow (Figure 4B). This suggests that thermal behavior is initiated at a lower T_{skin} compared to active cutaneous vasodilation. The initiation of thermal behavior during cold stress has been shown to temporally coincide with nearly maximal cutaneous vasoconstriction (38). In support of this observation, the T_{skin} Breakpoints for thermal behavior and skin blood flow during cold stress did not differ (Figure 4E). This suggests that the T_{skin} upon which nearly maximal cutaneous vasoconstriction is achieved is the same as that which initiates thermal behavior. It is worth noting that direct (e.g., pharmacological) evidence is

required to better determine the control of skin blood flow within the thermoeffector threshold zone and upon the decision to behaviorally thermoregulate. Moreover, it is unknown if the observed changes in skin blood flow directly contribute to the decision to behaviorally thermoregulate. There is no evidence that humans can consciously sense changes in efferent/sympathetic outflow. Therefore, we hypothesize that the impact of skin blood flow on the decision to initiate thermal behavior occurs via changes in local skin temperature or T_{skin} that occur subsequent to changes in skin blood flow (31). However, there is currently no direct evidence for such an arrangement.

Considerations

There are a few methodological considerations that warrant discussion. First, it is unknown if manipulating the temperature of the neck modifies the extent of autonomic thermoeffector activation. Local skin cooling can modify the extent of sudomotor activity during heat stress (8). However, the extent by which the temperature of the dorsal aspect of the neck can modify autonomic activation during heat or cold stress is unknown. We speculate that the extent by which changes in neck skin temperature may modify the extent of autonomic activation would be small compared to the extent of autonomic activation induced by whole-body heat or cold stress. This is qualitatively supported by a lack of change in the trajectories of the thermoeffector responses after neck skin temperature has been changed (Figure 2). Second, the findings of the present study are restricted to the methodological constraints employed herein. For instance, behavioral thermoregulation was restricted to controlling neck skin temperature. Thus, it remains unknown if our findings would be the same if more than one modality for thermoregulatory behavior had been allowed, as would occur in more real world settings. Moreover, the measurements of non-glabrous skin blood flow and sweat rate were localized to the upper arm. Changes in skin blood flow and sweat rate during heat stress are known to occur non-uniformly throughout the body (8, 45). Thus, it is unknown if these findings can be extended to other measurement locations, the whole-body, or glabrous skin. Furthermore, we used integrated laser Doppler flowmetry to provide a continual measure of skin blood flow. This technique samples from a relatively small skin surface area and does not measure absolute skin blood flow (21). Rather, laser Doppler measures changes in red blood cell flux, which is influenced by both changes in skin blood flow and vascularity under the area of interest. The laser Doppler probes were not moved during the experimental trials. Thus, we are confident that our red blood cell flux data are indicative of changes in local skin blood flow.

464 However, it remains to be seen if our findings would be consistent if absolute skin blood flow
465 were measured. Third, we measured T_{core} using a telemetric pill, an index of T_{core} that is slower
466 to change than esophageal temperature, but faster than rectal temperature (25). Thus, it is
467 likely that our measured changes in T_{core} were slower than those that would have occurred if
468 we measured esophageal temperature. This may have underestimated any potential
469 relationships between T_{core} and thermoeffector activation. However, we do not believe that this
470 is a major limitation because the primary aim of the study was to determine relationships
471 between thermoeffector activation relative to changes in T_{skin} not T_{core}. This was determined a
472 priori based on data indicating that when given the freedom to behaviorally thermoregulate,
473 behavior is elicited primarily by changes in T_{skin}, with minimal influence of T_{core}, whether
474 measured via esophageal or rectal temperature (39). Fourth, the current study utilized both
475 male and female subjects. We are underpowered to make formal comparisons between sexes.
476 However, differences are likely given that sex modulates autonomic temperature regulation
477 (13) and thermal perception (14, 15). Formal research is required to discern the effect of sex
478 on behavioral temperature regulation and interactions between autonomic and thermal
479 behavioral responses.
480
481 *Perspectives and significance*
482 The magnitude of forecasted heat stress in the coming decades is predicted to exceed the
483 limits of human autonomic temperature regulation (43). Thus, in the relatively near future
484 humans will become even more reliant upon behavior to regulate body temperature. However,
485 compared to our understanding of the control of autonomic thermoeffectors, our knowledge
486 regarding the mechanisms and modulators of thermal behavior is sparse (37). In this regard,
487 the present study provides fundamental insights indicating that thermal behavior is recruited in
488 coordination with autonomic thermoeffectors in healthy humans. Furthermore, although the
489 present study has identified a number of likely signals that mediate the decision to behaviorally
490 thermoregulate, research is required to directly examine the mechanisms by which the
491 decision to initiate thermal behavior is stimulated in both healthy and at risk populations. This
492 is important because there are a number of populations at particularly elevated risk of thermal
493 related morbidity and mortality that may also possess impairments in autonomic and
494 behavioral temperature regulation (e.g., older adults, etc. (23)). In fact, there is some, albeit
495 limited, evidence that thermal behavior is initiated at higher body temperatures in older adults
496 (11). Thus, there is a need for further research to understand the recruitment of

thermoeffectors, inclusive of behavior, in such at risk populations. Moreover, understanding the control of behavioral thermoregulation is particularly timely given the rapidly growing interest in thermal therapies, which require encroachment upon (33, 48, 49) or excursions beyond (3, 18, 30) the thermoeffector threshold zone to elicit benefits. Thus, knowledge regarding the mechanisms and modulators of thermal behavior may promote strategies for improving comfort and adherence to these thermal regimens to maximize the health benefits.

Conclusions

The present study demonstrates that during progressive heat stress increases in skin blood flow are elicited by the smallest increases in T_{skin} and that continued elevations in T_{skin} elicit thermal behavior and eventually sweating, the latter of which more closely coincides with changes in T_{core}. Moreover, during progressive cold stress decreases in skin blood flow are elicited by relatively small reductions in T_{skin}, while further drops in T_{skin} initiate thermal behavior. These findings suggest that during progressive heat and cold stress autonomic and behavioral thermoeffectors are recruited in coordination with one another and likely in an orderly manner relative to the comparative cost, with thermal behavior requiring more physiological resources compared to changes in skin blood flow, yet less than sweating or increases in metabolism.

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Tables

Table 1: Subject characteristics (Mean ± SD (range))[a]

Sex (M/F)	6 / 6
Age (y)	23 ± 2 (20 - 27)
Height (cm)	170 ± 8 (159 - 183)
Weight (kg)	70.2 ± 11.1 (52.2 - 86.9)
Body surface area (m^2)	1.8 ± 0.2 (1.6 - 2.1)
Body fat (%)	24 ± 14 (5 - 48)
Sum of skinfolds (mm)	135 ± 45 (61 - 213)
Resting heart rate (bpm)	59 ± 10 (46 - 80)
Resting systolic blood pressure (mmHg)	112 ± 9 (102 - 132)
Resting diastolic blood pressure (mmHg)	66 ± 6 (58 - 78)
Resting mean arterial pressure (mmHg)	81 ± 6 (73 - 96)
Physical activity (high/moderate/low) [b]	7 / 5 / 0
Montreal Cognitive Assessment Score [c]	29 ± 1 (26 - 30)

[a] Data from one female subject were excluded due to no evidence for behavioral thermoregulation. Hence, n=12. See text for further details. [b] stratified according to Craig et al. (9), [c] all subjects were in the normal range for age group: ≥26 (34).

Table 2: Slope1 and Slope2 during HEAT and COLD (mean ± SD).

	Slope1	Slope2
HEAT		
Neck skin temperature (°C/°C)	0.1 ± 0.1 [z]	-13.0 ± 19.0 [z]
Skin blood flow (PU/°C)	11 ± 9 [z]	232 ± 115 [z]
Local sweat rate (mg/cm^3/min/°C)	0.01 ± 0.01	1.71 ± 0.95 [z]
COLD		
Neck skin temperature (°C/°C)	-0.1 ± 0.4	3.4 ± 3.2 [z]
Skin blood flow (PU/°C)	-9 ± 7 [z]	-2 ± 2 [z]

[z] different from zero (P≤0.04).

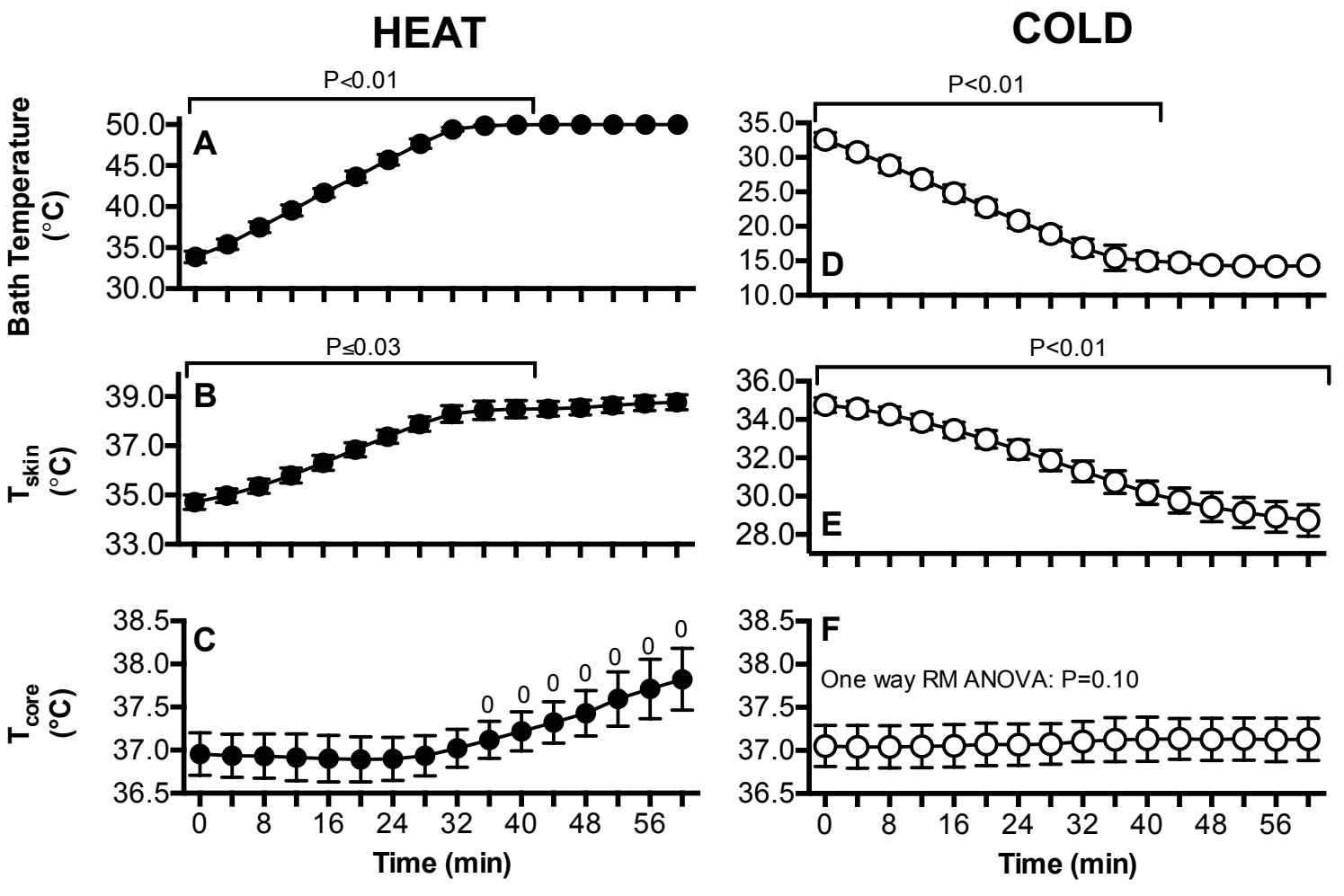
Figures

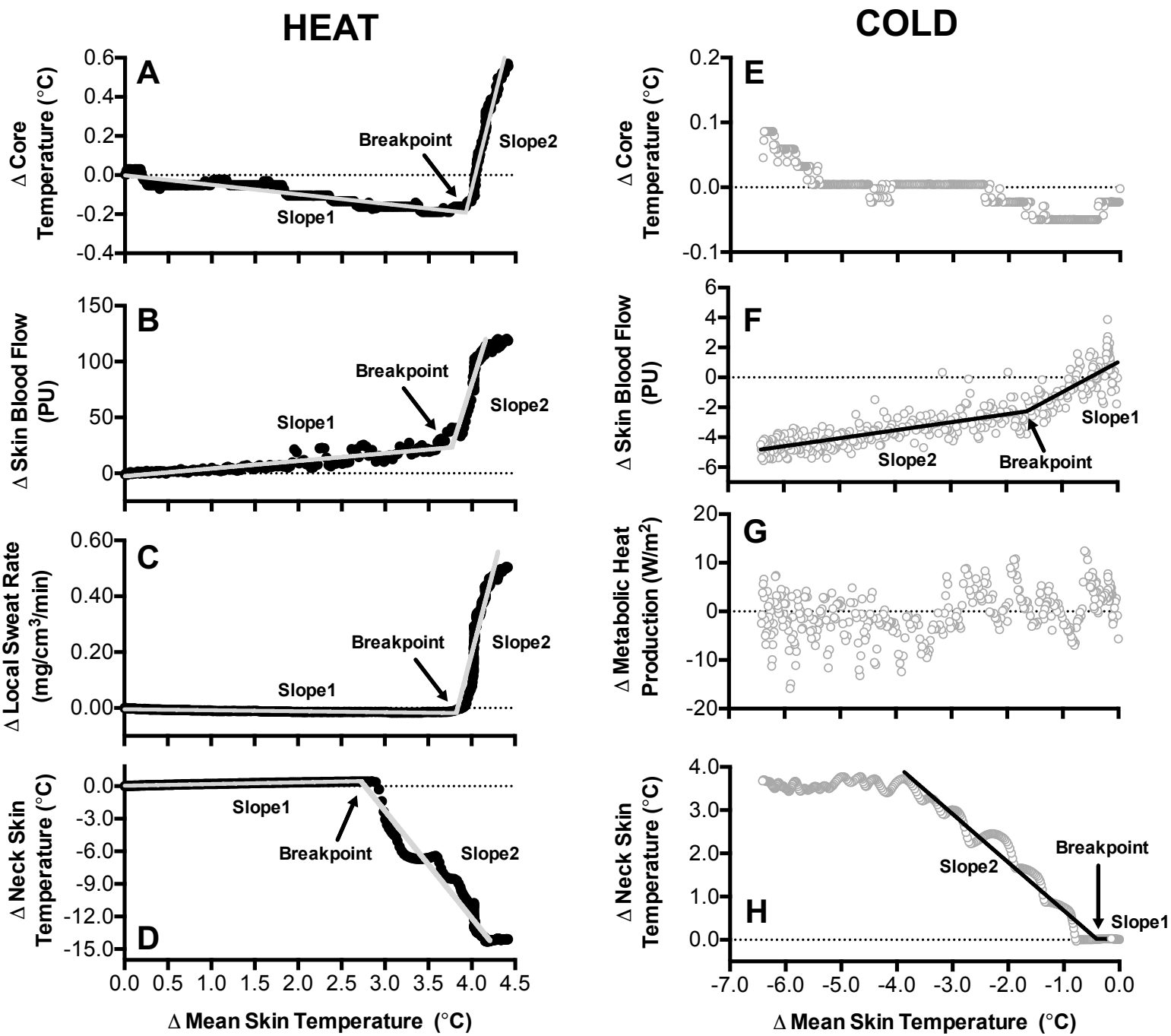
Figure 1: Water perfused suit bath temperature, mean skin temperature (T_{skin}), and core temperature (T_{core}) during progressive heat stress (HEAT, on left) and progressive cold stress (COLD, on right). Mean ± SD, n=12. All points underneath brackets are different from each other (P≤0.03). ^{0} different from 0 min (P≤0.02). P-value for not significant one way repeated measures (RM) ANOVA is noted.

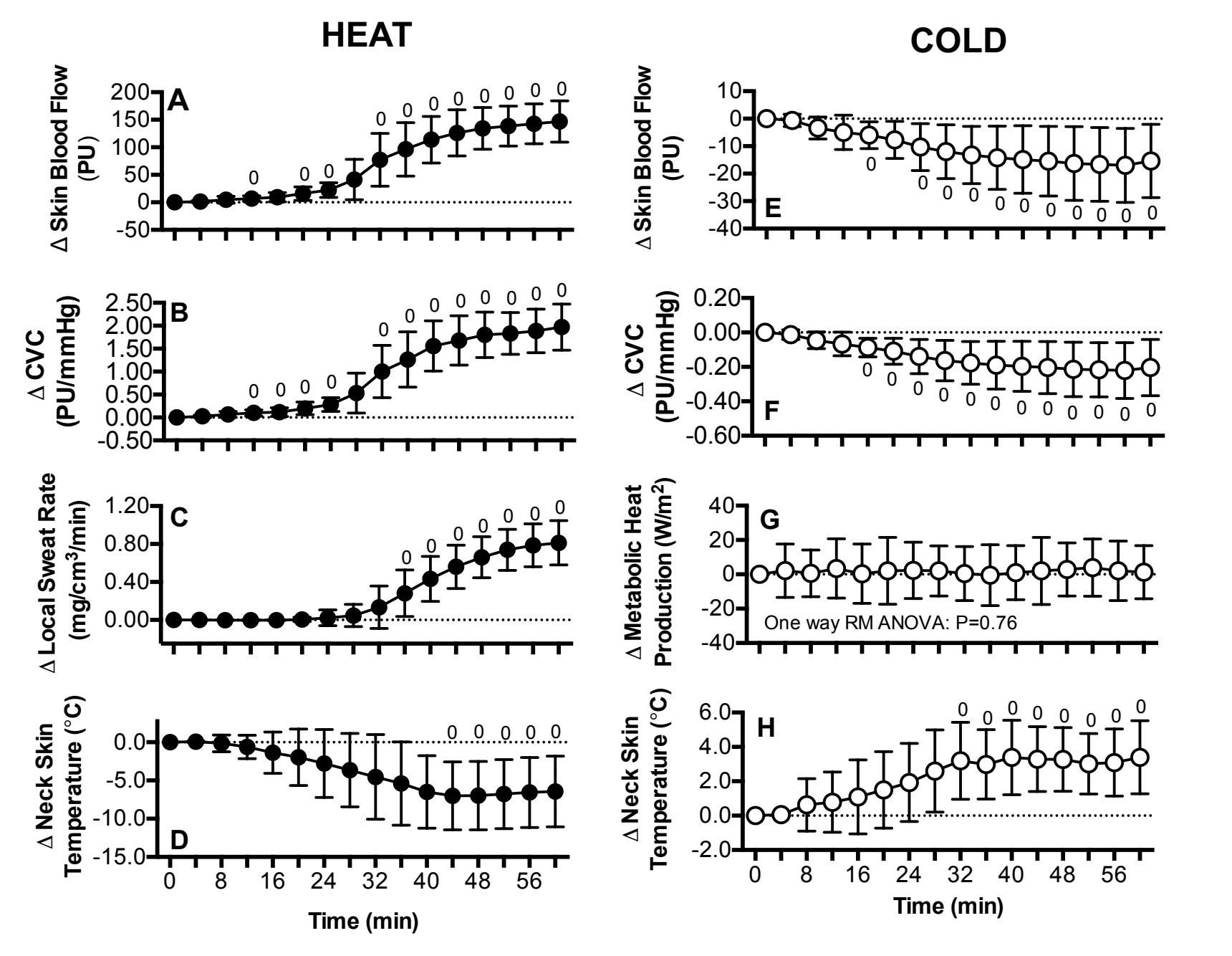
Figure 2: Typical tracing depicting changes (Δ) in core temperature and thermoeffector responses during progressive heat stress (HEAT, on left) and progressive cold stress (COLD, on right) as a function of changes in mean skin temperature, and the manner in the three parameters (Slope1, Breakpoint, Slope2) were identified via the segmental regression analysis.

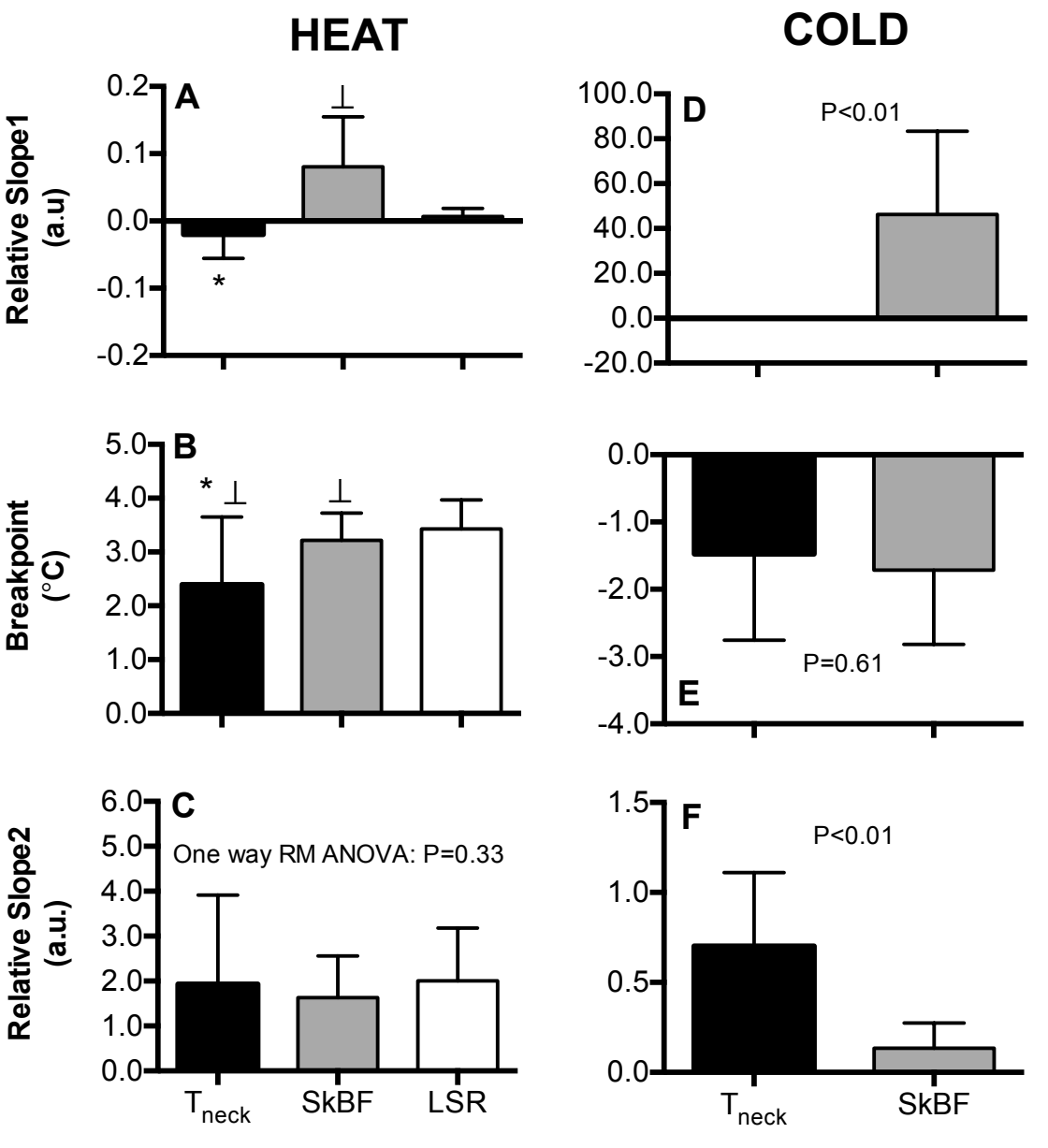
Figure 3: Average temporal changes in thermoeffector activation during progressive heat stress (HEAT, on left) and progressive cold stress (COLD, on right). Mean ± SD, n=12. ^{0} different from 0 min (P≤0.05). P-value for not significant one way repeated measures (RM) ANOVA is noted. CVC: cutaneous vascular conductance.

Figure 4: Relative Slope1, the Breakpoint, and Relative Slope2 for thermoeffector responses when determined relative to changes in mean skin temperature during progressive heat stress (HEAT, on left) and progressive cold stress (COLD, on right). Mean ± SD, n=12. $\perp$ different from local sweat rate (LSR, P≤0.04), * different from skin blood flow (SkBF, P≤0.04). P-value for not significant one way repeated measures (RM) ANOVA in HEAT is noted. P-values for paired t-tests in COLD are noted. T_{neck}: neck skin temperature.


HEAT
COLD
Bath Temperature (°C)
T skin (°C)
T core (°C)
P<0.01
P≤0.03
P<0.01
P<0.01
A
B
C
D
E
F
One way RM ANOVA: P=0.10
Time (min)
Time (min)


HEAT
COLD
A
B
C
D
E
F
G
H
Δ Core Temperature (°C)
Δ Skin Blood Flow (PU)
Δ Local Sweat Rate (mg/cm3/min)
Δ Neck Skin Temperature (°C)
Δ Metabolic Heat Production (W/m2)
Breakpoint
Slope1
Slope2
Δ Mean Skin Temperature (°C)


HEAT
COLD
Δ Skin Blood Flow (PU)
A
E
Δ CVC (PU/mmHg)
B
F
Δ Local Sweat Rate (mg/cm³/min)
C
Δ Metabolic Heat Production (W/m²)
G
One way RM ANOVA: P=0.76
Δ Neck Skin Temperature (°C)
D
H
Time (min)


HEAT
COLD
Relative Slope1 (a.u)
A
0.2
0.1
0.0
-0.1
-0.2
*
⊥
D
100.0
80.0
60.0
40.0
20.0
0.0
-20.0
P<0.01
Breakpoint (°C)
B
5.0
4.0
3.0
2.0
1.0
0.0
* ⊥
⊥
E
0.0
-1.0
-2.0
-3.0
-4.0
P=0.61
Relative Slope2 (a.u.)
C
6.0
5.0
4.0
3.0
2.0
1.0
0.0
One way RM ANOVA: P=0.33
T neck
SkBF
LSR
F
1.5
1.0
0.5
0.0
P<0.01
T neck
SkBF